

Review

Microbial lipids: Progress in life cycle assessment (LCA) and future outlook of heterotrophic algae and yeast-derived oils

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ABSTRACT

There is an increasing interest in the replacement of traditional plant based glyceride oils with microbial oils for fuel, food, dietary supplements and other pharmaceuticals and chemicals. To understand the environmental implications of this technology a systems approach is required. To date the majority of Life Cycle Assessment (LCA) studies have addressed the use of photoautotrophic (light stimulated growth, atmospheric carbon fixing) microbes to produce biofuels. But, with an increasing number of biotechnology companies using heterotrophic (non-light stimulated growth, requiring an organic carbon feedstock) microbes for large-scale industrial food and consumer goods production processes, it is important that the environmental implications of heterotrophic technology for products outside of the energy sector are better understood.

This review assesses the current life cycle literature available that relates to heterotrophic algae and yeast microbial oil production, evaluating where current gaps in knowledge and uncertainties lie. It also includes relevant LCA information from other production processes. Overall, the review finds significant gaps relating to both life cycle inventory and impact assessment information. Improved industrial data sets are needed, particularly for novel feedstocks, and cultivation and downstream processing towards non-fuel products. The implications of synthetic biology also need to be addressed. Impact assessment does not currently include a broad range of impact categories, focusing predominately on energy use and climate change. And to answer wider implications questions, further LCA studies are needed that take a more consequential approach. Addressing these areas is vital to ensure commercial viability, sustainability and public acceptance of the technology going forward.

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1. Introduction

There is an increasing interest in the development and commercialisation of microbial oils (MOs), also termed single cell oils (SCOs), due to growing concerns over the environmental land use impacts associated with first generation biofuels and plant oils for consumer products. Microbial oils are significant to both future fuel and food systems due to the similarity of some MOs to terrestrial oils and with the potential to directly replace them in both product systems.

In Europe, biofuels based on food-crops such as corn, soy or palm may be phased out within the European Union post-2020 through withdrawal of policy support and subsidy through the Renewable Energy Directive (RED) (2009/28/EC) (European Parliament and Council of the European Union, 2015). This will mean non-food based crops and microbial (microalgae/yeast/bacteria) sources will play a much greater part in the future biofuels mix beyond 2020. In order to fully understand how microbial oils can contribute to solving some of the challenges faced by the energy-food nexus it is important to understand their potential environmental impacts through a systems based approach.

Life cycle assessment (LCA) results have been routinely used to inform the debate on biofuels and the energy-food nexus. LCA has also been extensively applied to MOs, in particular to algae-derived oils. LCA can be used to evaluate the potential environmental impacts across the life cycle of the MO: from cultivation and fermentation of the microbe; through to extraction of the oil; downstream processing; and use. This process is shown in Fig. 1.

broken down for different types of microbes. LCA can be used to evaluate current process 'hotspots' (attributional) or model future wider technological effects (consequential). Almost all LCAs carried out on MOs to date have looked at the direct process related impacts from MOs, with none fully considering indirect effects (Collet et al., 2015).

The production process for MOs is dependent on the type of energy and carbon source required by the microbe. Phototrophs are able to utilise light energy to carry out metabolic processes. Often these are autotrophs which use carbon dioxide as a carbon source for the production of lipids, carbohydrates and proteins. Conversely, heterotrophs require organic carbon, often in the form of simple sugars, in order to grow and produce a range of more complex organic carbon compounds. Most LCA studies to date have focused on photoautotrophic algae for the production of biofuel. This is despite the increasing commercial use of heterotrophic algae and yeast for the production of a wide range of products. For example, *TerraVia* use algae fed by sugarcane, corn and stover, sugar beet, switchgrass, and other forest and waste residues to produce oils for food, personal care, and aquaculture and animal nutrition. *Amyris* use yeast to produce a range of industrial lubricants, personal care products, and demonstrated the use of yeast to manufacture the anti-malarial compound *artemisinin* (Paddon et al., 2013). It is important to understand the potential benefits and challenges associated with this type of heterotrophic MO process which utilises organic carbon sources, as opposed to fixing carbon from a carbon dioxide source and using photon energy for growth, or the utilisation of plant-derived oils such as palm oil (Fig. 1).

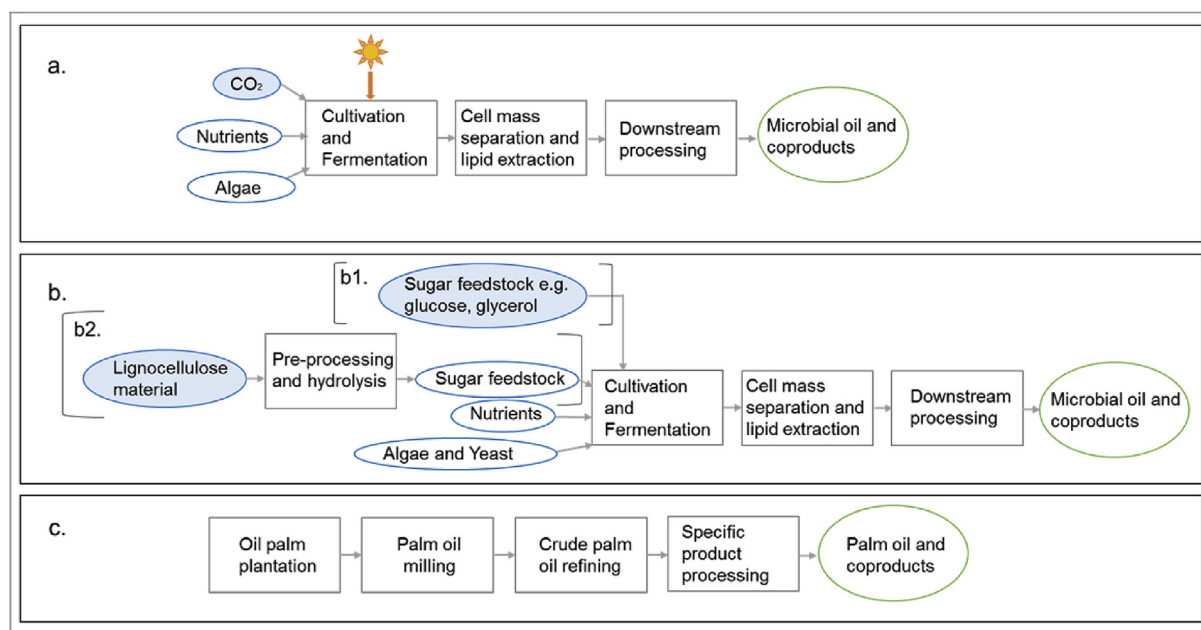


Fig. 1. Oil production: a. microbial oil production via photoautotrophic algae, b. microbial oil production via heterotrophic algae and yeast (b1 utilising a simple sugar source such as glycerol, b2 utilising a more complex lignocellulose source which requires pretreatment), c. palm oil production process.

This study evaluates the current gaps in environmental LCA and techno-economic analysis of microbial oils from the perspective of heterotrophic algae and yeast, making recommendations for future LCA work in the area. This is important both in understanding how microbial oils can contribute to advanced biofuel production under EU RED and US Renewable Fuel Standard (RFS) (EPA, 2010), and the implications of microbe use for a wide range of food, personal care and other high-value compounds. An outline of oleaginous microbes and their uses is given in Section 3. The use of heterotrophic algae and yeast presents a number of methodological challenges for LCA which will be discussed further in sections 4 and 5.

2. Methodology

Life cycle assessment (LCA) is a technique which allows comparative life cycle evaluation of product system. Variations on LCA such as Life Cycle Costing (LCC) and process Techno-Economic Analysis (TEA), can be applied at various stages of the life cycle to evaluate the most cost effective methods for raw material production, product and coproduct processing or end-of-life management. These techniques can work in tandem with an environmental LCA to evaluate technical, economic and environmental challenges, which is particularly useful when evaluating new technologies such as this.

The majority of LCA research to date has focused on the production of lipids from photoautotrophic algae for biofuel applications. As a result, LCA and TEA papers relevant to heterotrophic algae and yeast are reviewed from across all areas of microbial oil production. This is broken down across each production stage in Section 4. For initial *feedstock production* papers from heterotrophic algae and yeast were used. However, there have been few heterotrophic studies carried out. This stage is not comparable with photoautotrophic algae oil production which uses CO₂ as its carbon feedstock. Information for this production stage was also supplemented with recent bioethanol LCAs, as the feedstock preparation approach for bioethanol is the same as for heterotrophic algae and yeast. For *Cultivation and Fermentation* only heterotrophic algae and yeast LCAs and TEAs were reviewed here. For *cell mass separation and lipid extraction* both heterotrophic and photoautotrophic LCA/TEA studies can be used as the method to break the cell wall in order to extract the oil, and the extraction of the oil itself are comparable between the two. For *downstream processing* both heterotrophic and photoautotrophic LCA/TEA studies can be used as both processes are comparable. This is also the same for the *use phase*. Table 1 shows the production stages and relevant life cycle inventory information at each stage. LCA studies chosen for this review were selected based on their provision of a functional unit, system boundaries and methodology consistent with LCA (but not necessarily a specified accordance with ISO 14044). All studies reviewed were LCAs except Braunwald (2013), Koutinas et al. (2014) and Jena et al. (2015) which were TEA studies, used to supplement the low numbers of heterotrophic algae and yeast studies. Additional bioethanol LCAs from Prasad et al. (2016) and Sebastiao et al. (2016), and TEAs from Baral and Shah (2016) and Johnson (2016) are also discussed in this analysis. A breakdown of studies reviewed by year is given in Fig. 2.

3. Oils and high-value compounds from microbes

Micro-organisms that have been investigated for the purposes of lipid production include yeast, algae, fungi and bacteria. Oleaginous microbes can convert substances such as sugar, carbon dioxide and organic acids to single cell oils, usually under stress conditions such as nutrient depletion. Once the intracellular lipids have been produced they can be removed from the cell through

solvent, mechanical, or enzymatic extraction techniques (Sitepu et al., 2014). Oil and hydrocarbon content within lipid producing microbes can range between 20 and 60%.

3.1. Industrial cultivation

Microalgae are often cultured in open circular or raceway ponds. These tend to be cheaper to construct and more durable than sophisticated closed photobioreactors (PBRs). However, raceway ponds can have a number of disadvantages including; controlling the culture temperature, insufficient CO₂ delivery or light delivery and water loss due to evaporation (Liu et al., 2014). PBRs also face challenges at higher production volumes in achieving efficient light dispersion, and come at a greater initial investment cost than raceway ponds.

Due to characteristics such as fast growth, high cell density, and high oil productivity, heterotrophic algae systems are of increasing interest. The most commonly studied of these are species from the genus *Chlorella*. Heterotrophic microalgae can utilise a range of carbon sources including sugars, hydrolysed carbohydrates, waste molasses, acetate and glycerol. By not requiring light to grow, using heterotrophic algae can make the production process for microbial oil simpler (Liu et al., 2014) (Perez-Garcia et al., 2011). Despite this, opinion on the potential for large scale cultivation of heterotrophic algae is mixed (Miao and Wu, 2006; Perez-Garcia et al., 2011; Tabernero et al., 2012). Perez-Garcia et al., 2011 state that microalgae cultivation for large-scale industrial processes such as biofuels production is problematic due to current cultivation technology making it uneconomical. This is a challenge faced by both photoautotrophic and heterotrophic production systems, and is why many LCA and TEA papers point to the benefits of utilising co-products to obtain greater economic value from the process (Chowdhury et al., 2012; Grierson et al., 2013; Khoo et al., 2013; Koutinas et al., 2014; Quinn et al., 2014; Orfield et al., 2015).

3.1.1. Lipids and co-products

Yeasts are another promising alternative to photoautotrophic algae for the production of biofuels and other high value compounds. Yeasts can produce a wide variety of lipid including fatty acids, acylglycerols, sterol esters, free sterols, other hydrocarbons such as carotenoid pigments which can be used in food additives and supplements, long chain alcohols, waxes, polyprenols and isoprenoid quinones (Sitepu et al., 2014).

Yeasts cannot break down lignocellulosic materials themselves in order to access the sugars they contain, feedstocks require a prior treatment step before they are metabolised by the yeast. This often involves an initial pretreatment step followed by enzymatic hydrolysis. Pretreatment can be a mixture of biological, chemical or physical, with subsequent enzymatic breakdown using a mixture of cellulose, hemicellulose pectinase and ligninase (Sitepu et al., 2014).

3.1.2. Conditions for lipid production

Draaisma et al. (2013) identify a number of specific requirements for sustainable and cost effective food commodity products from microbial sources. These include a need to consider the type of photobioreactor used, location, species, water source, medium source, process strategy, downstream processing and biorefinery. Important sustainability indicators include water use, land use, land use change, medium use, eutrophication, global warming and energy demand. Also included in their assessment are aspects such as consumer acceptance, stability and processability.

3.1.3. Regulation of edible oils

Regulation affecting the use of yeast oil as an edible oil as

Table 1

Breakdown of life cycle information from heterotrophic and phototrophic studies including papers reviewed for each life cycle stage.

Production/life cycle stage	Microbial oil life cycle information		Reviewed papers
	Heterotrophic studies (organic carbon, no photon stimulated growth)	Photoautotrophic studies (carbon fixation from CO ₂ , photon stimulated growth)	
Feedstock preparation	Included	Excluded	Braunwald et al. (2016), Chang et al. (2015), Koutinas et al. (2014), Summers et al. (2015), Jena et al. (2015), Orfield et al. (2015), Baral and Shah (2016), Prasad et al. (2016), Sebastiao et al. (2016), Chowdhury et al. (2012), Karlsson et al. (2016)
Cultivation and fermentation	Included	Excluded	Braunwald et al. (2016), Chang et al. (2015), Cok et al. (2013), Koutinas et al. (2014), Summers et al. (2015), Jena et al. (2015), Orfield et al. (2015), Karlsson et al. (2016)
Cell mass recovery and lipid extraction	Included	Included	Lardon et al. (2009), Baliga and Powers (2010), Batan et al. (2010), Clarens et al. (2010), Sander and Murthy (2010), Stephenson et al. (2010), Clarens et al. (2011), Brentner et al. (2011), Collet et al. (2011), Campbell et al. (2011), Khoo et al. (2011), Hou et al. (2011), Shirvani and Yan (2011), Soratana and Landis (2011), Benemann et al. (2012), Chowdhury et al. (2012), Davis et al. (2011), Frank et al. (2012), Resurreccion et al. (2012), Soratana et al. (2012), Vasudevan et al. (2012), Yanfen et al. (2012), Gao et al. (2013), Grierson et al. (2013), Soratana et al. (2014), Ventura et al. (2013), Zaimes and Khanna (2013), Ajayebi et al. (2013), Khoo et al. (2013), Liu et al. (2014), Braunwald et al. (2016), Woertz et al. (2014), Fortier et al. (2014), Adesanya et al. (2014), Perez-Lopez et al. (2014), Ponnusamy et al. (2014), Collet et al. (2015), Handler et al. (2014), Quinn et al. (2014), Koutinas et al. (2014), Beal et al. (2015), Connelly et al. (2015), Bennion et al. (2015), Summers et al. (2015), Jena et al. (2015), Gnansounou and Raman (2016), Manganaro and Lawal (2016), Monari et al., 2016
Downstream processing	Included	Included	Lardon et al. (2009), Baliga and Powers (2010), Batan et al. (2010), Clarens et al. (2010), Sander and Murthy (2010), Stephenson et al. (2010), Clarens et al. (2011), Brentner et al. (2011), Collet et al. (2011), Campbell et al. (2011), Khoo et al. (2011), Hou et al. (2011), Shirvani and Yan (2011), Soratana and Landis (2011), Benemann et al. (2012), Chowdhury et al. (2012), Davis et al. (2011), Frank et al. (2012), Resurreccion et al. (2012), Soratana et al. (2012), Vasudevan et al. (2012), Yanfen et al. (2012), Gao et al. (2013), Grierson et al. (2013), Silva et al. (2015), Soratana et al. (2014), Ventura et al. (2013), Zaimes and Khanna (2013), Ajayebi et al. (2013), Khoo et al. (2013), Liu et al. (2014), Braunwald et al. (2016), Woertz et al. (2014), Fortier et al. (2014), Adesanya et al. (2014), Perez-Lopez et al. (2014), Ponnusamy et al. (2014), Collet et al. (2015), Handler et al. (2014), Quinn et al. (2014), Koutinas et al. (2014), Beal et al. (2015), Connelly et al. (2015), Bennion et al. (2015), Summers et al. (2015), Jena et al. (2015), Gnansounou and Raman (2016)
Use – Oil and coproducts	Included	Included	Davis et al. (2011), Benemann et al. (2012), Zaimes and Khanna (2013), Khoo et al. (2013), Fortier et al. (2014), Connelly et al. (2015)

opposed to use as a biofuel feedstock will be different. Wakelyn and Wan (2004) discuss oil extraction processes in the context of US regulation. They state that extractive solvents such as *n*-hexane, which has been approved by the FDA for use many food products but not necessarily as an extractive solvent, is often described as incidental additives in that 'it is present but does not have any technical or functional effect' (Wakelyn and Wan, 2004). These incidental additives are generally exempt from labelling but must be used in accordance with FDA good manufacturing practices. In the EU, directive 2009/32/EC on the use of extraction solvents for foodstuff and food ingredients, sets limits for the levels of hexane that can be present as 1 mg/kg. In the US, for direct food substances affirmed as generally recognised as safe (GRAS) for cocoa butter substitutes like palm oil 90 percent of the oil should be triglyceride with no more than 1 percent monoglyceride. Total glycerides should account for 98 percent. Colour should be clear, bright and free from suspended matter. Residual hexane or methanol should be present in less than 5 parts per million, and residual fatty acid ethyl esters present in no more than 20 parts per million. There is no maximum residue limit (MRL) for hexane isomers (Wakelyn and Wan, 2004). This means that far more stringent measures are required within the steps for downstream processing of heterotrophic algae and yeast-derived lipids for edible oils than may be required for biofuels.

This type of downstream processing has not been considered in

LCA studies to date, and therefore little is known on the additional impacts this may have in terms of energy use on the production process. Processing of edible oils often involves a refining step in which free fatty acids and other phospholipids are removed, a subsequent bleaching step to remove colour-producing substances, and a final further refining step to remove any volatile compounds (associated with undesirable flavours or odours). Deodorisation is performed at temperatures of 245–275 °C, and a negative pressure of 2–10 mm Hg (Wakelyn and Wan, 2004). This area needs far greater development through LCA and TEA to determine potential impact from this type of downstream processing.

4. Manufacture and life cycle of heterotrophic algae and yeast products

With much of the LCA data produced to date focused on photoautotrophic algae, there have been very few LCAs focused on heterotrophic algae and yeast-derived oil. This is not reflective of the current level of commercial application for this type of microbial process. Seemingly, there is also a disconnection between publication focus on microbial oil production for biofuel applications and what can be seen happening within the commercial world as microbial oil producers' shift towards food and personal care products. Some of the challenges identified in evaluating the environmental implications of heterotrophic algae and yeast

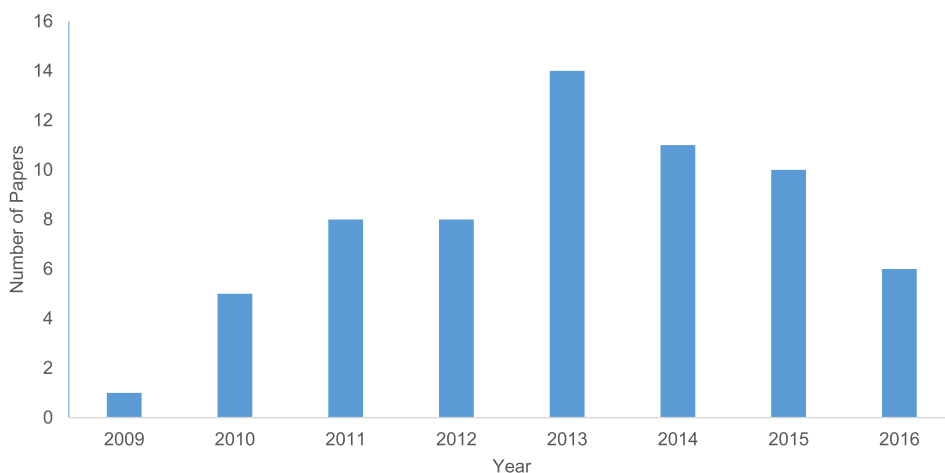


Fig. 2. Publication by year of phototrophic and heteroautotrophic microbial oil papers reviewed.

derived oils are given in Fig. 3.

A major challenge in applying LCA towards heterotrophic microbes for use within food and cosmetics is the lack of industrial data on the production processes. This relates to cultivation and fermentation, cell mass recovery and extraction and downstream processing. As discussed by Dunn et al. (2016) patent information can often be used to guide modelling of production processes, but often patents lack key, more specific, data relating to processing conditions, solvent use, yield, and production efficiencies which makes accurate representation of a commercial system through LCA difficult.

The technology has potential impacts on several areas. The

market for co-products may displace other products, with energy products potentially substituting primary process energy production. Studies to date are typically foreground process focused (attributional), with some choosing to avoid allocation or analysis of their coproducts through substitution or system expansion (Stephenson et al., 2010; Clarens et al., 2011; Benemann et al., 2012; Chowdhury et al., 2012; Resurreccion et al., 2012; Gao et al., 2013; Orfield et al., 2015). LCA studies predominantly represented functional units in terms of energy provision – the production or combustion of 1 MJ or BTU of biodiesel. This makes for comparison between algal biodiesel production, but makes it difficult to evaluate microbial oil use within other non-energy product systems. All

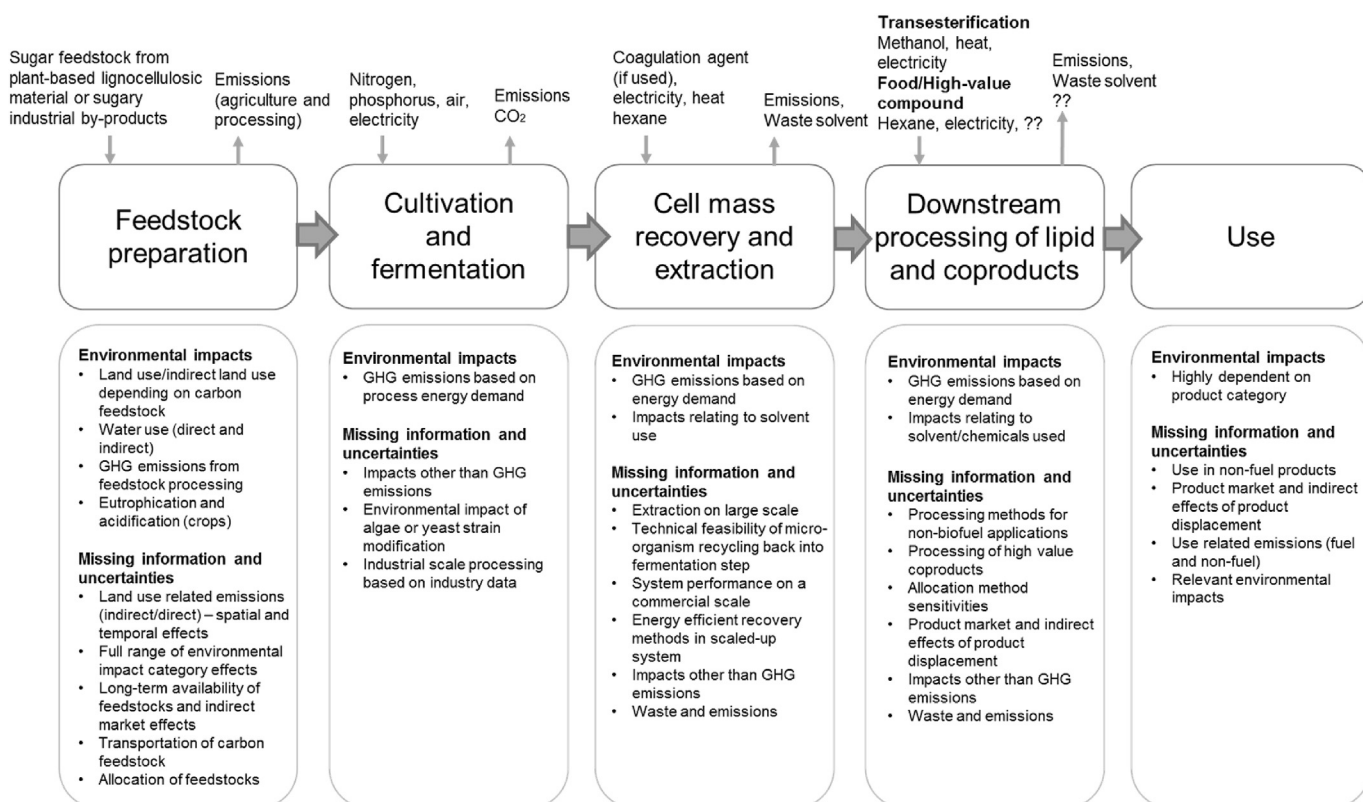


Fig. 3. Production of heterotrophic microbial oils: current LCA uncertainties and challenges.

Table 2
Heterotrophic algae and yeast LCA studies.

Study	Feedstock	Cultivation	Cell mass recovery and extraction	Functional unit	System boundary	Environmental indicators	Major contributors to GHG emissions	Total GHG emissions
Chang et al., 2015	Glycerol	0.2 ML bioreactor	Gravity settling and centrifugation followed by homogenisation and hexane extraction	Combustion of 1 MJ biodiesel	Well-to-wheel	GHG emissions/energy	Glycerol, aeration/mixing	71 gCO ₂ e/MJ (glycerol) 17 gCO ₂ e/MJ (molasses)
Orfield et al., 2015	Sugarbeet/Sugarcane	Cylindrical reactor	Centrifugation, followed by pressure homogenisation and wet hexane extraction	Production of 1 L of biodiesel	Well-to-tank	GHG emissions, water stress, occupied land, energy, indirect land use change	Feedstock, aeration/mixing	4 kgCO ₂ e/litre of biodiesel
Summers et al., 2015	Delactose whey permeate	50 L bioreactor	Centrifugation, HTL (300 °C, 18–20 MPa)	Production of 1 MJ biodiesel	Well-to-tank	GHG emissions/energy	HTL process	30 gCO ₂ e/MJ
Karlsson et al., 2016	Wheat straw	Cylindrical reactor	Pressure filtration to 70% moisture, homogenisation, hexane extraction	Production of 1 MJ biodiesel	Well-to-tank	Energy use		

but one heterotrophic LCA reviewed in this study evaluated impact in terms of energy production (well-to-tank or well-to-refinery) or combustion (well-to-wheel).

Indirect effects are also important when evaluating carbon feedstock, particularly for heterotrophic microbes utilising organic carbon compounds from a variety of different sources. There have been no consequential LCAs conducted so far on microbial oil technology. Few studies have addressed a full range of environmental impact areas, with the majority focusing on energy and global warming potential. The limitations of only addressing global warming potential and energy use have also been raised in recent review papers on phototrophic technology (Collet et al., 2015; Quinn and Davis, 2015). For heterotrophic algae and yeast LCAs, Orfield et al., 2015 address spatial impacts through evaluation of water stress and indirect land use change. Karlsson et al. (2016) evaluate energy aspects only. For phototrophic studies there are a range of impact categories and impact assessment models used. All impact modelling across phototrophic and heterotrophic studies is done at midpoint. Where a range of impact areas have been assessed the most commonly used models are CML (Lardon et al., 2009; Hou et al., 2011) and TRACI – particularly for US-centric studies.

Tables 2 and 3 summarise heterotrophic algae and yeast LCA and TEA studies. Fig. 3 details the potential environmental impacts, missing information and uncertainties relating to the production of yeast derived oil and its coproducts adapted from microbial oil literature review. Given the production process for heterotrophic algae and yeast oil is similar to that for algae oil it is appropriate to draw on the findings of algae LCA. The process is split into 5 areas: (1) Feedstock preparation (2) cultivation and fermentation, (3) cell mass recover and extraction, (4) downstream processing of lipid and coproducts, and (5) use. Each area represents different challenges and uncertainties.

4.1. Feedstock preparation

Heterotrophic feedstocks can be simple sugary compounds such as molasses or glycerol, or can be more complex lignocellulosic material which is pre-treated and hydrolysed before being added as a carbon feedstock during cultivation and fermentation.

Summers et al. (2015) provided an LCA and techno-economic evaluation of using delactosed whey permeate as a growth medium for yeast *Cryptococcus curvatus* to produce lipids for biodiesel. The delactosed whey permeate can be obtained as a low-value product from the processing of milk. It contains lactose, calcium, potassium and magnesium making it a useful growth media for the yeast (Summers et al., 2015). Their bio-refinery model using hydrothermal liquefaction (HTL) assumed a capability to process 3.8 million gallons of dairy effluent per day. This would require a large scale dairy processing facility supported by a number of farms. This study does not include indirect impacts from diverting use of the delactose whey permeate in other uses, assuming it is used on the land, as cattle feed or discharged to a waste water treatment facility (Summers et al., 2015).

Koutinas et al. (2014) evaluate the economics of yeast oil production for biodiesel. They assume the use of glucose as a carbon source for the yeast *Rhodospiridium toruloides*. Overall, this TEA study finds glucose to be almost 80% of the total raw materials cost. Chowdhury et al. (2012) combine phototrophic and heterotrophic processes using the production of glycerol in the transesterification processing of phototrophic algae oil as a feedstock for further heterotrophic fermentation. Chang et al., 2015 also use glycerol as a feedstock. This was economically allocated based on glycerol as a by-product from biodiesel production. They cite a 2.42:1 ratio of

Table 3
Heterotrophic algae and yeast TEA studies.

Study	Feedstock	Assumptions	Major operational economic impacts	Cost
Jena et al., 2015 Koutinas et al., 2014	Delactose whey permeate Glucose from lignocellulose/ waste material	Yeast, HTL Conversion Yeast, industrial plant production at 10,000 t/y. Conversion to glucose excluded	Taxes, HTL process, inoculation Electricity	US\$3.5 per gallon US\$5.5 per kg oil, US\$5.9 per kg biodiesel
Braunwald et al., 2016	Glucose from lignocellulose/ waste material	Yeast, conversion to glucose excluded	Maintenance, general expenses (taxes, research)	US\$2350 per ton (closed reactor) US\$1723 per ton (open-pond reactor)

carbon source (glycerol) to algae. Chowdhury et al., 2012 also look at substituting glycerol for molasses, as a by-product from sugar production. They found that substituting glycerol for molasses reduced upstream emissions by 77–81%.

All of the studies discussed in this section so far have focused on obtaining simple sugars as by-products for other industrial processes. Another source of carbon feedstock is from plant-based material. Orfield et al. (2015) consider two plant-based feedstocks – sugarcane and sugarbeet. They found that the best performing scenario used sugarcane, where the bagasse produced as a crop residue was combusted within a high pressure co-generation steam cycle system to produce electricity onsite. This offsets demand for grid electricity for the production process. Because sugarbeet does not have any combustible high energy by-products, under none of the scenarios evaluated in the study was a sugarbeet carbon source favourable to conventional diesel (Orfield et al., 2015).

Karlsson et al. (2016) use wheat straw as their carbon feedstock. The straw is pretreated through steam explosion and then hydrolysed enzymatically. Total sugar recovery from wheat straw is 619 g per kg straw. The solid fraction (containing lignin) is fed into a CHP with the power generated used in the production process and excess sold back to the grid.

For information on the conversion of lignocellulosic material to less complex sugary compounds, the production process for bioethanol is a useful analogous process. A number of recent TEA and LCA studies have investigated this process to evaluate the most economic/environmentally beneficial route to deliver monosaccharides for bioethanol production. The National Renewable Energy Laboratory (NREL) also have a number of published techno-economic studies which include energy analysis (Humbird et al., 2011). Baral and Shah (2016) use pretreatment within an ionic liquid followed by enzyme hydrolysis. This was evaluated in the context of a number of different lignocellulosic feedstocks. The study found the cost of the ionic liquid, its recovery and the recovery of heat from the process to be key factors to determining economic viability. The authors also raise concern over the availability of feedstocks such as switchgrass and poplar in making the process viable. Johnson (2016) found that by integrating the enzyme treatment process so that the same feedstocks for cellulase and ethanol are used could decrease cost of production by up to 20%.

Prasad et al. (2016) contrasted different pretreatment methods. They found that pretreatment method had a large impact on overall GHG emissions. Using a dilute acid led to GHG emissions far higher than applying a pretreatment approach that using high temperature and pressure liquid water. Sebastiao et al. (2016) used waste pulp and paper sludge to produce bioethanol via neutralisation with hydrochloric acid and enzyme hydrolysis. For almost all environmental impact categories the neutralisation and enzyme hydrolysis step contributed to over 60% of total impact. The only categories this was not the case for were photochemical oxidation, acidification and eutrophication where downstream processing and concentration steps took a greater share of the environmental impact.

Uncertainties relating to feedstock preparation for heterotrophic microbes include the upstream processing data for plant-based carbon sources, which currently relies on sufficiently aggregated bioethanol data sets (Fig. 3). The range of carbon feedstocks available makes this particular part of the production process, far more complex to model than a comparator photoautotrophic process. Where data sets from the production of bioethanol are used, the impacts need to be understood in context of heterotrophic microbial oil production. This is important in contrasting heterotrophic and photoautotrophic processes, and also evaluating the viability of heterotrophic technology against incumbent processes. The use of sugary industrial by-products such as delactosed whey permeate or glycerol may require less preprocessing before it can be used as a feedstock for algae or yeast, but careful consideration is needed as to how these are allocated, and what the indirect effects are on existing markets for these products. These aspects have so far not been fully considered. For more complex media, further work is needed to understand the conditions under which the use of plant-based material such as sugarcane, sugarbeet, corn etc. can be environmentally and economically viable, and the role that waste lignocellulosic material plays within this mix. For waste plant material, again, allocation and indirect impacts are important to explore in greater depth. This relates particularly to the use of waste agricultural material in animal feed or other product streams and as a retainer of soil quality.

These factors lead to parametric uncertainties for land use (direct and indirect) change impact relating to different geographic contexts (Fig. 3). This has only been evaluated so far by Orfield et al. (2015) for heterotrophic microbes, but given the range in both crop plants and waste materials used in the commercial production of heterotrophic microbial oil (TerraVia 2016), more work is needed within this area. There are also broader data requirements for impact categories other than global warming potential. The majority of studies focus only on GHG emissions and energy use, whereas a wider range of potential impact areas need to be assessed. These include eutrophication, acidification, land use, and water use, often associated as being key impact areas for agricultural/crop production systems, along with human health and ecosystem health impacts.

4.2. Cultivation and fermentation

The carbon feedstock enters the process during the cultivation and fermentation steps. Here heterotrophic algae and yeast require nitrogen, phosphorus, and oxygen alongside the carbon feedstock. Unlike phototrophic microbes, heterotrophs do not require light provision, which can be a significant energy user in the photoautotrophic algae oil production process (Perez-Lopez et al. (2014)). This makes the fermentation process for phototrophs and heterotrophs different. Table 2 summaries the findings from heterotrophic algae and yeast LCAs.

Orfield et al. (2015) evaluated 3 different aeration scenarios within their cylindrical fermenter model, with a power provisions

of 1, 2 and 3 kW/m³. They found that 1 kW/m³ and 2 kW/m³ scenarios gave an overall better fossil energy ratio (FER) than conventional diesel. However, the 3Kw/m³ base case scenario gave a FER which was 2-fold higher than fossil diesel. Of the aeration power input 40% of this went on mixing, 60% on aeration. Chang et al. (2015) used a 0.2 ML industrial bioreactor for their process. They obtained an energy return on energy invested (ERoEI) of 0.5 (indicating twice the energy needed per unit of energy produced for use) for their baseline process which was attributed to the running of the impeller during fermentation, along with the carbon source feedstock (glycerol) and other extraction processes. Given that these studies are some of the few to cover heterotrophic algae LCA it is important that further research is conducted. This is particularly important as several large biotechnology companies utilise heterotrophic fermentation for their microbial oil production.

Adesanya et al. (2014) looked at reactor and process design and found that Airlift PBR reactors used less electricity but required more fertiliser than raceway ponds. Overall, raceway ponds led to fewer GHG emissions. Summers et al., 2015 found that the growth phase contributed significantly to both the operational and capital costs for the process. Fermentation was less significant in terms of GHG impacts due to applied renewable fuel and nitrogen recycling credits to the process. Beal et al., 2015 suggested improvements to make the production process more commercially viable. These include the production of high value coproducts, improving biomass productivity to over 25 g/m² per day, automation to reduce labour costs, the integration of biofuel production with the production of renewable energy, the use of atmospheric or waste forms of carbon and nutrients, and an improvement in downstream processing. Braunwald et al. (2016) found that heterotrophic microbial oils cannot yet compete with plant oils due to high capital costs relating to the fermenter and efficiencies of nutrient and wastewater recycling. Koutinas et al. (2014) also found fermentation contributed significantly to overall operational and capital costs, and advised towards the isolation and adaption of robust yeast strains and innovative fermenter designs in order to reduce costs.

4.2.1. Synthetic biology and genetic modification

The need for robust yeast and algae strains in order to make the production process economically viable raises uncertainties in the assessment of environmental impacts associated with novel synthetic biology techniques. A number of studies have attempted to model the scale up of MO production, but there is little modelling information available for yeast and even fewer industrial data sources. Obtaining industrial production data of yeast oils and their coproducts is important in being able to make an accurate and fair comparison with existing incumbent processes.

The environmental implications of genetic modification is an area which is poorly covered by traditional LCA studies. This is an area of research which is increasingly important given the rise of synthetic biotechnology in recent years. In this instance, the genetic modification of the yeast strain is important to ensuring economic production of the lipid and coproduct, and resistance to inhibitors and other factors which may reduce product yields. The modification of yeast strains is typically carried out through a process of directed evolution. Laboratory directed evolution involves the optimising or altering the activities of individual genes (Packer and Liu, 2015). This speeds up the process of selective breeding, meaning that positive traits for a specific application can be enhanced by the manipulation of single genes.

Other very recent methods for genome engineering include CRISPR (clustered regularly interspaced palindromic repeats)

which is based on a naturally occurring bacterial defence system that enables the targeting of specific parts of genetic code and editing of DNA at specific locations. The CRISPR sequences hold DNA information that the bacteria have copied from invading viruses which are then transcribed into RNAs and guide a Cas (CRISPR associated) protein to cut matching viral DNA preventing it from replicating. Learning from this bacterial system, the principles can be utilised with the Cas protein which is modified to both cut at specific DNA locations and also to turn gene expression on at specific locations. The technology has many promising applications in medicine and cancer treatment, but could also be more widely applied within the biotechnology sector (Gaj et al., 2013; Doudna and Charpentier, 2014).

As genome engineering and synthetic biology play a wider role in biotechnology, particularly in relation to improving the ability of microbes to produce specific substances for fuels, food, pharmaceuticals, cosmetics or other high value chemicals under a range of conditions, the life cycle implications for this type of engineering need to be understood. Within the broader application of life cycle thinking two distinct areas might cover some of the challenges faced by synthetic biology but neither are adequately covered by traditional LCA methodology. These are the socio-political and environmental implications of the technology. As with other genetic engineering systems such as GM life cycle discussion generally tends to be limited to the societal implications of the technology as a function of social acceptance. The environmental implications of synthetic biology and the engineering of genetic systems is rarely discussed within the bounds of life cycle assessment as this falls outside the current capabilities of LCA. The combination of life cycle thinking with risk assessment practice in this area could at least provide a partial picture of potential implications for ecosystems in the event of environmental release, as has been suggested for other emerging technology systems such as nanomaterials (Grieger et al., 2012), but this remains a novel area of investigation with significant further research required (Fig. 3).

For cultivation and fermentation there needs to be greater clarity on the implications of a wide range of environmental impact categories. Studies are focused on GHG emissions given the high energy demand at this process stage and importance placed on GHG emissions by regulatory frameworks. More work is needed to understand, for example, the implications of fertiliser use to provide nitrogen and phosphorus to the system.

Given that the majority of LCA and TEA studies suggest ways in which the cultivation process can be further improved to make the process more commercially viable, data from heterotrophic microbial oil producers is important in understanding what can be achieved at an industrial scale. Given the small number of heterotrophic algae and yeast studies in this area, data based on what can currently be achieved by industry is key in being able to understand how heterotroph MO and photoautotroph MO compare, and how this compares with the production of conventional vegetable oils like palm oil for food, fuel and other high-value compounds.

4.3. Cell mass recovery and extraction

Cell mass recovery and extraction involves the recovery of lipid from within the algae or yeast cells, and of any coproducts produced from the biomass. Methods for harvesting MO biomass include flocculation using an aluminium or iron coagulating agent (Clarens et al., 2010; Stephenson et al., 2010; Campbell et al., 2011; Khoo et al., 2011) or through gravity settling (Benemann et al., 2012; Resurreccion et al., 2012; Gao et al., 2013; Quinn et al., 2014). This is then followed by a centrifugation step (Baliga and

Powers, 2010; Batan et al., 2010; Clarens et al., 2010; Stephenson et al., 2010; Davis et al., 2011; Frank et al., 2012; Vasudevan et al., 2012; Ajayebi et al., 2013; Grierson et al., 2013; Ventura et al., 2013). Cells are often then homogenised (Batan et al., 2010; Gao et al., 2013; Soratana et al., 2014) and extracted using hexane (Lardon et al., 2009; Sander and Murthy, 2010; Brentner et al., 2011; Campbell et al., 2011; Hou et al., 2011; Khoo et al., 2011; Soratana and Landis, 2011; Yanfen et al., 2012; Ventura et al., 2013; Zaimes and Khanna, 2013). For yeast, Braunwald et al. (2016) suggest a straight centrifugation step with further dewatering through spray drying. Hexane is then used to extract the lipid from the yeast cells.

Koutinas et al., 2014 suggest the additional use of a homogeniser to mechanically disrupt the yeast cells in order to extract the lipid. They assume the hexane is recycled to reduce operating cost and environmental impact. In their production process a fluidised bed dryer is used in place of spray drying due to the larger scale of biodiesel production in their study. Manganaro and Lawal 2016 find the drying step to be the greatest contributor to CO₂ emissions in their MO oil production process. Monari et al., 2016 used scenario analysis to evaluate the use of supercritical CO₂ instead of hexane to extract the MO. They found combinations using supercritical CO₂ and a waste CO₂ carbon source had the lowest climate change impacts; however, the use of waste CO₂ as a carbon feedstock had a far greater impact on carbon emissions than extraction method. Ubando et al., 2015 found oil extraction was by far the highest energy using step in the MO production process regardless of cultivation system used (Ubando et al., 2015). This is confirmed in earlier work by Lardon et al. (2009), which found that 90% process energy relates to oil extraction. For Lardon et al., 2009 this largely relates to the amount of energy required for drying, highlighting some of the challenges around applying a dry hexane solvent recovery technique, and points to the need for further development in wet extraction and solvent recovery.

Much of the work cited above looks at the laboratory scale production of MOs. Here methods for extraction are likely to overestimate/show highly varied energy demand and solvent requirement due to lack of any industrial scale process optimisation and solvent recycling (although solvent recycling is considered in a number of studies). The main environmental impacts are likely to come from the process energy demand from drying and extraction (mechanical) and impacts relating to the use of hexane or other solvents in the extraction process, with the contribution this has related to optimisation of the process and solvent recycling (Fig. 3).

There are a number of scenarios that have been investigated relating to the cell recovery and extraction of lipid. Chang et al. (2015) applied gravity settling, before centrifugation and homogenisation. The lysed cell solution was then mixed with hexane, and a hexane stripper used to recover the solvent back from the process. Both homogenisation and hexane extraction steps contributed to over 30% of the total GHG emissions associated with their process. Sander and Murthy (2010) contrasted the use of centrifugation with a filter press for the first step of their dewatering process. They found overall the filter press had the lower energy requirement and lower carbon emissions. Finally, as mentioned previously Monari et al., 2016 evaluated the use of supercritical CO₂ instead of hexane. Given the variety of techniques that can be employed in the recovery and extraction of lipids from MOs that relate to yeast, more data is needed on how these are applied at an industrial scale. This includes more information on waste and emissions from the process. Also needed is more data on the recycling of components such as solvents and even algae or yeast cells that do not yet contain sufficient lipid content within them. The broader environmental implications of hexane use and other coagulation agents needs to be ascertained.

4.4. Downstream processing of lipid and coproduct

Downstream processing of lipid and coproduct is often considered in less depth within LCA studies. Braunwald (2013) investigated the feasibility of carotenoid production alongside yeast oil production. Here segregated production of the two components was found to be more favourable. Carotenoids are common high value pigments found in most photosynthetic organisms. These can be used in dietary supplements or as food additives due to their powerful antioxidant properties. Other economically valuable compounds include (algal) polysaccharides, other polyunsaturated fatty acids such as DHA which is a typically found in fish oil, and a range of other chemicals.

Common coproducts considered in MO LCA studies are oilcake, solid digestate and glycerol (Lardon et al., 2009; Baliga and Powers, 2010; Sander and Murthy, 2010; Shirvani and Yan, 2011; Frank et al., 2012; Resurreccion et al., 2012; Soratana et al., 2012; Vasudevan et al., 2012; Quinn et al., 2014; Collet et al., 2015). Baliga and Powers 2010 consider the recovery of algae meal for use in animal feed (Baliga and Powers, 2010). Sander and Murthy (2010) include the recovery of carbohydrate and protein coproducts, with the carbohydrate fraction substituting corn ethanol production (Sander and Murthy, 2010). Stephenson et al., 2010 assume the algae residue would be used to produce methane by anaerobic digestion. Glycerol is also a coproduct in the process, and it was assumed it would be used to manufacture soap. Ventura et al. (2013) assumed that oilcakes produced would be used in anaerobic digestion, where excess heat substitutes natural gas use. Here glycerol is also used to substitute heat production. In Zaimes and Khanna (2013) glycerol was used instead to substitute the production of glycerol derived from fossil fuels.

Chang et al. (2015) contrasted the use of hydroprocessing or transesterification to produce biodiesel. Transesterification reacts the MO with alcohol to obtain a biodiesel which contains FAME and glycerol. The hydroprocessing step removes oxygen, nitrogen and other heteroatoms to yield a hydrocarbon product that does not contain any aromatics and therefore must be blended with conventional diesel to meet requirements (Chang et al., 2015). Overall, they found that using a molasses feedstock and using the hydroprocessing method this was the only scenario that gave an energy return on energy investment (MJ MJ⁻¹) greater than 1 (the energy invested is less than the energy return from the fuel). Orfield et al. (2015) assumed the transesterification of the MO, and anaerobic digestion of the algal biomass. The anaerobic digestion process provided heat and electricity back to the site, substituting grid electricity use. The transesterification process energy was found to be a fraction of the energy required in other prior stages such as fermentation.

A challenge in the LCA of yeast oil is modelling the downstream processing of the lipid where biofuel application is not the end use, and the modelling of the numerous potential coproducts (Fig. 3). Most of the LCA studies on MO evaluated in this paper assumed the use of their coproducts in the application of energy or heat production. Here often economic or energetic allocation is used. For Stephenson et al., 2010 allocation of coproducts is complicated by the application of their coproducts in different market areas, with algae residue used in anaerobic digestion and glycerol in soap production. Because of this a hybrid approach is applied with algae residue allocated through substitution and glycerol through market price. Given the range in coproduct production and applications it is clear there is no one size fits all approach to allocation in this instance. Allocation via system expansion in the MO LCA studies reviewed is through a process of substitution, where coproducts such as oilcakes are used to produce energy which is then fed back into the local electricity mix. System expansion to include

coproducts which are not used to create energy was not considered within the scope of the majority of LCAs reviewed.

Expanding the analysis to include coproduct systems is an area requiring further investigation. Because of the variety of coproducts able to be produced from microbial sources, which are often at varying stages of commercial interest, this presents an area of significant uncertainty in modelling commercial demand and displacement of other product systems. In this situation a number of scenarios should be constructed to represent possible revenue and environmental impact streams, including a consideration towards how the use of these coproducts displaces existing products.

Little is known on the additional impacts production for food or consumer goods may have from an LCA perspective (Fig. 3). As discussed in Section 3, the processing of edible oils may involve a refining step in which free fatty acids and other phospholipids are removed, a subsequent bleaching step to remove colour-producing substances, and a final further refining step to remove any volatile compounds (associated with undesirable flavours or odours). Future LCA research is required to understand how these processes influence the overall GHG emissions and other environmental impacts associated with MO production, and how this compares with the production of vegetable oils or other incumbent products MOs and coproducts replace.

4.5. Use of the final MO product

A number of photoautotrophic algae studies have included use of the energy product within their studies scope (Davis et al., 2011; Benemann et al., 2012; Khoo et al., 2013; Zaimes and Khanna, 2013). For many of these studies emissions savings relating to the combustion of the biodiesel are considered directly within context of the overall process, making granularity and therefore transparency on the relative combustion emissions values for different GHGs difficult. Coproduct use for energy products are most commonly included when using the remaining microbial biomass within anaerobic digestion. Fortier et al. (2014) and Connelly et al. (2015) Connelly et al., 2015 consider the use of production of aviation fuel from algae. Fortier et al., 2014 found that combustion contributed to far less than 1% of total GHG emissions. Connelly et al., 2015 found that once you consider non-CO₂ combustion emissions the fuel only gives a 25% reduction on conventional aviation fuel due (making it less attractive under the Renewable Fuels Standard). They conclude that a far greater GHG saving can be attained by using MO in land-based transport fuel rather than aviation fuel (based on the multipliers attached to atmospheric combustion).

Aside from the use of MO within transport fuel, the use of algae and yeast derived oils has been poorly considered for other applications in a life cycle context. Perez-Lopez et al., 2014 was the only study reviewed to evaluate algae for the production of high value chemicals. They assessed the production of the antioxidant carotenoid Astaxanthin, however, they excluded final processing of the carotenoid product from their study. As stated previously in Section 3, further LCA investigation is needed to understand use in the context of products outside biofuels.

5. Recommendations and future outlook

Heterotrophs as a source of oil for fuel and food products show great potential in reducing reliance on high biodiversity land for palm and soybean oil. While MOs will not displace all edible oils in the short to medium term, their continued development and implementation could curtail the associated deforestation and land-use impacts associated with the cultivation of

these crops. As research in this area continues MOs have the potential to compete with terrestrial oils on cost and environmental impacts, particularly when environmental impact from land-use is included.

It is important to understand the environmental implications of this technology better in order to ensure the most sustainable pathway forward. LCA studies have predominately focused on phototrophic algae derived oils which follow similar but not the same processes of production. Whilst these studies have not necessarily been consistent, they have all pointed to challenges faced by high energy demand at various points in the production process leading to an often unfavourable comparison with conventional diesel and first generation biodiesel. What has been made clear from the LCA research so far is the need for strong integration of waste feedstocks, waste water, and improved heterotroph productivity (oil and biomass), process efficiency and drying methods/extraction from wet biomass.

Further LCA research is needed in the area of MO for applications outside its use in biofuels, particularly where MO has applications in the food industry. Understanding how MOs can contribute to the interlinked challenges of clean fuel and food production is important in understanding wide-ranging applications and contribution to sustainable development this technology can make. The lack of LCA development in this area to date is due to lack of scaled-up facilities and infancy of the microbial-derived edible oils market. Based on the challenges and uncertainties shown in Fig. 3, many of these can be solved through demonstration of the technology at scale, incorporating sensitivity and scenario analysis into LCA study design. Issues such as those relating to the environmental impacts associated with synthetic biology and CRISPR may never be adequately assessed through LCA alone, but could be considered alongside LCA using other more suitable assessment methods. Steer on how this is done can be taken from how life cycle information is used to assess other emerging technology systems such as nanomaterials.

There are several ways in which LCA can be used to advance MO technology as a whole, and to specifically contribute to increasing our understanding of the potential for heterotrophic MOs to directly replace vegetable oils like palm oil.

1. Assist the improvement of heterotrophic microbial oil production process

There has been a significant amount of LCA research over the years on microalgae-derived biofuels, but little investigation using LCA for heterotrophs. Lessons can be learnt from the results of microalgae studies as the production process shares many similarities; however, more specific research on heterotrophic microbes is required. LCA benefits algae and yeast-specific process development through using the results to aid design and direction of research. This is beneficial from initial research and development stages through to pilot and full-scale production.

2. Understand life cycle implications of microbial oils and their coproducts used in a range of fuel, food, and high-value compounds

Despite significant research into applications of oils and other products derived from microbes, there is little LCA research into the environmental implications of microbial oil outside its use in biofuel. Given that the majority of industrial MO production includes the use of lipid, protein and other coproducts in a range of food and non-food consumer goods, further research is needed to understand how MOs compare against incumbent technologies in these areas.

3. Develop industry partnerships and improve database information on MOs for fuel and food

Access to industry data and communication with industry is vital during the initial stages of new technology emerging. Finding appropriate LCA inventory data on the scaled-up production of heterotrophic yeast and microalgae is important in obtaining realistic environmental impact values for the process. As with (1) above, this data can be used to serve as a baseline from which performance can be improved.

4. Fill missing gaps in evaluating a range of environmental impact areas

Whilst some authors have included a range of impact categories within their LCAs for phototrophic microbes, most have limited impact evaluation to climate change and energy use. LCA can include other wide ranging impacts from eutrophication, ecotoxicity, human toxicity, acidification, photochemical ozone formation, ozone depletion, respiratory organics, land use, water use, and resource depletion. A fuller spectrum of environmental impacts need to be addressed.

5. Evaluation of MOs indirect impacts and consequential effects for a rigorous understanding of the technology's implications

Whilst attributional LCA may be useful in determining initial processing parameters and guiding design of specific processes in comparison with a reference system, evaluating the wider implications of the technology is equally important particularly when using the results to inform broader decision-making. This of course introduces significant uncertainty, particularly in modelling several different coproduct impacts within different product sectors. Currently, there are few studies on MOs (phototrophic or heterotrophic) which consider consequential effects. Despite the uncertainties, this is particularly important to understand the consequences of oil and coproduct production and use, and the total full share of background production systems that are affected.

6. Conclusions

Microbial oils derived from heterotrophic algae, yeast and bacteria are likely to play a large part in the future production of biofuel, further chemicals and potentially as a new food source. This would help limit further biodiversity and land use impact associated with the growing demand for terrestrial oils. Up until now life cycle assessment (LCA) focus has been predominately on the use of phototrophic algae-derived oil within fuels. Given the recent shift towards MO use in food, consumer goods, supplements and medicines by biotechnology companies it is essential that the environmental implications of MOs application within these products is understood. Overall, this is a promising technology whose application across a wide number of food and non-food products is likely to increase. Current LCA research points to the cultivation being the greatest contributor to GHG emissions, but results are highly linked to feedstock choice in terms of their performance relative to conventional diesel. Further work is needed in this area, and there are significant knowledge gaps particularly relating to novel feedstock preparation, the impact of genetic modification, cultivation on a commercial scale, and downstream processing for non-fuel applications. Further industrial partnerships and LCA development is important to take better account of non-fuel applications.

Key points

- LCA of heterotrophic algae and yeast is constrained by lack of data on industrial production. This is especially true for feedstock preparation and cultivation stages
- Key sensitivities include the modelling and allocation of feedstocks, the allocation of coproducts and byproducts, and the overall energy efficiency of the production system
- Assessment must include a wider range of environmental impacts alongside GHG emissions and energy demand
- Future LCAs need to consider wider implications of microbial biotechnology for a range of application areas including in-direct effects

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